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TITLE: Genetic Susceptibility to Prostate Cancer Among Ashkenazi Jews

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13. ABSTRACT (Maximum 200 Words)
This project will test the basic hypothesis that a given microsatellite marker allele occurs with greater frequency among the individuals affected with prostate cancer than among the controls. These studies will take advantage of the fact that two populations of Ashkenazi Jewish men are readily available for a case-control study. The first is a group of men at high heritable risk based on their having early-onset prostate cancer. The second is a group of men at low heritable risk who have no personal or family history of prostate cancer. Thus, we expect to observe predisposition alleles in the men at high risk that are not present in the men at low risk. The predisposition genes are likely to be within chromosomal regions in which loss of heterozygosity has occurred. Because these regions have remained identical by descent since the high-risk mutations occurred, they can be recognized by the presence of specific alleles of microsatellite markers in the high-risk group that are not present in the low-risk group.

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INTRODUCTION

This study used several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. It was based on the observations that 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been endogamous causing them to have more genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population' s endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). Ashkenazi Jews represent such a population.

BODY

Task 1. Subject identification. Months 1-24

Samples from 200 high-risk subjects were identified during the first year. To increase the power of the study, samples from an addition 100 cases were identified during the second year. The medical histories of each of these subjects were reviewed, confirming ethnicity and diagnosis of prostate cancer, and noting family history, age at diagnosis and Gleason score at time of diagnosis. For each subject, tissue blocks were obtained from non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections were cut. DNA was purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized. This technique reproducibly amplified the DNA samples 50-fold. From the pool of low-risk subjects, an additional 200 samples were chosen and added to the 200 samples already obtained for subsequent analysis. These were amplified using PEP for subsequent analysis. Methods of drying down these DNAs in 96-well microtiter plates for subsequent use were developed.

Task 2. Development of markers. Months 13-24

A. Markers from regions associated with loss of heterozygosity (LOH) in prostate cancer were identified and fluorochrome-labeled primers were synthesized. We identified microsatellite markers for each of the following chromosomal regions 1q24-q25, 7q31, 8p21-p22, 10q23-q25, 13q14, 16q22, 17p, 17q21-q22, Xq11-q13. We added markers to our analysis. Because of uncertainties about relative map positions, we confined our markers to those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (http://www.ncbi.nlm.nih.gov/GeneMap99) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998). Recently, we remapped some of these markers using the annotated human genome map (http://genome.ucsc.edu). We

discovered that the average spacing of our markers was 2 Mb, i.e. at the density that we originally planned (table 1). However, some of the markers did not map to the chromosomes to which they were originally assigned. These were excluded from subsequent analysis.

B. Standard PCR conditions will be developed for each of these markers. The primer sequences for each of these markers was identified using standard databases (http://www.gdb.org). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). Methods for multiplex analysis were developed to enhance the throughput of marker analysis. For each fluorochome, three markers (small, medium, and large) were coamplified in the same well. The net effect of this grouping was that multiple markers could either be amplified simultaneously and/or pooled from separate amplifications for subsequent fragment length analysis to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions were optimized. This led the analysis of up to 9 markers simultaneously in a single run.

Table 1: Revised Microsatellite Positions Based on Human Genome Annotation

Marker	Chr	Position	Segmental difference	Mean segmental difference	Marker	Chr	Position	Segmental difference	Mean segmental difference
D1S243	1	2.01	3.99		D8S264	8	2.12	1.55	
D1S2870	1	6.00	0.67		D8S262	8	3.66	2.54	
D1S214	1	6.67	0.38		D8S1742	8	6.20	0.30	
D1S2694	1	7.05	4.15		D8S277	8	6.50	2.31	
D1S2667	1	11.20	2.15		D8S351	8	8.81	0.46	
D1S228	1	13.35	0.77		D8S503	8	9.27	1.32	
D1S407	1	14.12	0.27		D8S520	8	10.59	0.72	
D1S507	1	14.39	4.07		D8S265	8	11.32	1.44	
D1S2644	1	18.46	0.97		D8S552	8	12.75	0.09	
D1S199	1	19.43	0.55		D8S1106	8	12.85	1.84	
D1S2843	1	19.98	1.09	1.73	D8S511	8	14.69	0.14	
D1S478	1	21.07			D8S1827	8	14.83	0.42	
D1S2844	1	160.14	5.14		D8S1731	8	15.25	0.41	
D1S2799	1	165.28	2.43		D8S549	8	15.66	0.96	
D1S452	1	167.71	1.24		D8S254	8	16.62	3.22	
D1S2815	1	168.95	2.79		STS-M15856	8	19.83	0.54	
D1S218	1	171.74	3.58		D8S258	8	20.38	1.05	
D1S212	1	175.32	1.55		D8S282	8	21.43	0.19	
D1S215	1	176.87	0.48		D8S560	8	21.61	0.84	
D1S2883	1	177.34	0.56		D8S136	8	22.46	0.00	
D1S117	1	177.90	1.64		D8S1786	8	22.46	0.23	
D1S466	1	179.54	2.43		D8S1752	8	22.69	0.13	
D1S2127	1	181.98	2.81		D8S1734	8	22.82	2.02	
D1S518	1	184.79	0.22		SHGC-6135	8	24.84	5.58	
D1S222	1	185.01	0.38		D8S339	8	30.41	3.27	
D1S238	1	185.39	3.28		D8S283	8	33.69	1.67	
D1S422	1	188.67	3.36		D8S87	8	35.36	0.11	
D1S2757	1	192.03	3.88	2.24	D8S1750	8	35.47	2.25	
D1S413	1	195.91			D8S1722	8	37.72	2.18	
D1S213	1	?			D8S255	8	39.90	1.36	
D1S2827	1	213.20	1.01		D8S268	8	41.26	10.93	2.12
D1S490	1	214.21	2.81		D8S587	8	52.20		
D1S2758	1	217.01	2.20		D8S1130	?			
D1S2871	1	219.21	9.54		D8S133	?			
D1S251	1	228.75	0.32		D8S201	?			
D1S2709	1	229.07	2.70		D8S261	?			
D1S446	1	231.76	1.17		D8S298	?			
D1S235	1	232.93	0.74						
D1S2850	1	233.67	3.99						
D1S180	1	237.66	0.26						
D1S2785	1	237.92	1.90						
D1S2842	1	239.82	0.82						
D1S2811	1	240.64	3.18	2.36					
D1S2836	1	243.82							

Marker	Chr	Position	Segmental difference	Mean segmental difference
D10S195	10	76.96	0.94	
D10S202	10	77.91	2.29	
D10S219	10	80.20	5.24	
D10S1658	10	85.44	4.22	
D10S541	10	89.66	0.82	
D10S1739	10	90.48	3.56	
D10S583	10	94.03	0.82	
D10S185	10	94.85	1.95	
D10S571	10	96.80	2.35	
D10S1709	10	99.15	1.60	
D10S198	10	100.74	1.36	
D10S192	10	102.10	1.94	
D10S1697	10	104.04	0.95	
D10S222	10	104.98	1.53	
D10S1671	10	106.52	4.38	
D10S597	10	110.90	2.09	
D10S1682	10	112.99	3.32	2.31
D10S562	10	116.30		
D10S205	12	48.06		
D10S532	?			
D10S1644	?			
D16S398	16	65.91	7.94	
D16S512	16	73.85	2.45	
D16S515	16	76.30	1.62	
D16S518	16	77.92	0.78	
D16S3049	16	78.70	0.12	
D16S3096	16	78.82	0.08	
D16S516	16	78.90	0.04	
D16S504	16	78.95	0.49	
D16S3040	16	79.43	0.42	
D16S507	16	79.86	2.84	
D16S422	16	82.69	3.61	
D16S520	16	86.30	0.60	
D16S476	16	86.89	0.78	
D16S413	16	87.68		1.67
D16S402	?			
D16S289	?			
D16S347	?			

Marker	Chr	Position	Segmental difference	Mean segmental difference
D17S938	17	6.45	0.00	
D17S796	17	6.45	1.01	
D17S960	17	7.46	1.55	
D17S786	17	9.01	28.51	
D17S250	17	37.53	1.90	
D17S800	17	39.43	1.15	
D17S776	17	40.58	1.00	
D17S855	17	41.58	0.03	
D17S1323	17	41.61	0.24	3.89
D17S1327	17	41.85	3.48	
D17S791	17	45.33		
D17S1810	?			
D17S856	?			
DXS1047	X	127.78	8.23	
DXS1062	X	136.01	1.07	
DXS1192	X	137.07	0.91	
DXS1232	X	137.99	0.21	
DXS1205	X	138.20	1.30	
DXS1227	X	139.50	1.38	
DXS8106	X	140.88	1.82	
DXS8043	X	142.70	0.21	
DXS8028	X	142.91	1.50	
DXS1200	X	144.41	1.06	
DXS548	X	145.47	0.84	
DXS6687	X	146.31	0.78	
DXS1193	X	147.09	0.55	
DXS8011	X	147.64	3.00	
DXS8061	X	150.64	2.65	1.61
DXS1108	X	153.29	0.22	
DXS1107	X	153.51		
DXS8103	?			
DXS1177	?			
DXS1113	?			

Task 3. Data analysis. Months 24-48

Genotyping for each individual in the study is being performed for each of the markers. Allele calling is performed using the ABI genotyper software. Whenever possible, genotypes for multiple markers, each tagged with a different fluorochrome are run in the same lane to conserve on the number of electrophoretic runs. Each lane contains internal controls for calibration. Samples with alleles of known size were included in each run to validate the calibration. In addition, one or more samples were typed in duplicate in each run to assess the reproducibility of results. Each of the microsatellites is called independently by at least two individuals and entered into a spreadsheet. If the results are discordant, they meet to attempt to resolve these discordances. When not possible, the analyses are repeated.

Standard calculations of odds ratios using contingency tables are performed for cases and controls to estimate the risks associated with each marker (table 2). Allele frequencies were identified to determine where the risks might be spurious based on small sample sizes (Figure).

Task 4. A final report will be written. Months 36-48.

The most interesting results were observed for X chromosomal markers suggesting the possibility of one or more susceptibility loci on that chromosome. Significant associations were observed at DXS1205/allele 180 (138.20 Mb), DXS1227/allele 170 (139.20 Mb), DXS548/allele 200 (145.47 Mb), DXS8061/alleles 135 and 141 (150.64 Mb), highlighted by arrows in the attached figure.. If replicated in another data set from this or another population (such as the Netherlands Cohort Study on Diet and Cancer that we have collected), this study will provide additional evidence for an X linked locus, as suggested previously (Xu, et al., 1998).

Table 2: Odds Ratios for X Chromosomal Markers

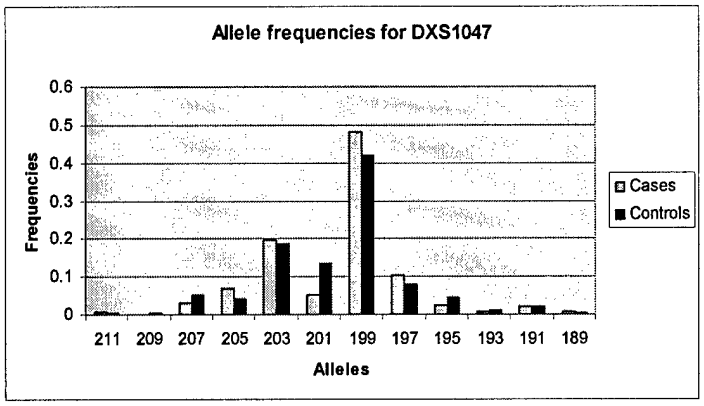
Allele	Odds ratio	lower 95% confidence interval	higher 95% confidence interval
DXS1047			
211	1.64	0.15	18.16
209	0.00	--	--
207	0.59	0.22	1.56
205	1.76	0.83	3.74
203	1.08	0.69	1.70
201	0.34	0.16	0.73
199	1.29	0.90	1.84
197	1.34	0.73	2.47
195	0.56	0.19	1.63
193	0.54	0.06	4.53
191	0.89	0.24	3.22
189	3.28	0.20	52.71
DXS1108			
175	1.60	0.71	3.60
173	1.75	1.19	2.59
171	0.59	0.39	0.90
169	0.82	0.33	2.05
167	0.00	--	--
165	7.02	0.63	77.98
163	3.50	0.49	25.09
DXS1193			
138	0.00	--	--
136	0.59	0.24	1.41
134	0.99	0.57	1.72
132	1.56	0.90	2.73
130	0.70	0.27	1.83
128	1.73	0.20	15.07
126	0.00	--	--
DXS548			
208	--	--	--
206	0.00	--	--
204	8.74	0.78	97.54
202	2.90	0.48	17.62
200	*3.04*	*1.20*	*7.68*
198	0.71	0.08	5.98
196	8.74	0.78	97.54
194	0.00	--	--
192	1.08	0.43	2.72
190	1.28	0.78	2.13
188	0.12	0.03	0.50

Allele	Odds ratio	lower 95% confidence interval	higher 95% confidence interval
DXS8106			
282	*18.82*	*4.15*	*85.29*
278	5.08	0.45	56.75
276	0.29	0.08	0.98
274	0.00	--	--
272	0.67	0.18	2.46
270	0.00	--	--
268	3.94	1.08	14.31
266	0.00	--	--
264	1.68	0.28	10.22
262	0.97	0.59	1.59
260	0.15	0.04	0.66
258			
256	0.00	--	--
DXS1113			
174	*6.56*	*1.08*	*39.69*
172	1.02	0.56	1.86
170	*2.37*	*1.14*	*4.89*
168	0.00	--	--
166	1.47	0.96	2.24
164	0.40	0.16	1.04
158	0.00	--	--
154	0.00	--	--
152	0.89	0.60	1.32
150	1.07	0.12	9.67
DXS1205			
194	0.00	--	--
192	0.39	0.09	1.71
190	0.91	0.34	2.46
188	0.33	0.04	2.57
186	1.01	0.28	3.62
184	1.46	0.52	4.12
182	0.58	0.39	0.87
180	*1.78*	*1.21*	*2.61*
178	--	--	--
176	--	--	--
DXS1227			
200	0.00	--	--
188	0.00	--	--
186	0.68	0.15	3.02
184	0.00	--	--
182	0.35	0.08	1.52
180	0.00	--	--
178	0.84	0.47	1.51
176	0.86	0.39	1.88
174	1.85	0.87	3.95
172	1.07	0.61	1.88
170	*3.08*	*1.98*	*4.78*

Allele DXS8061	Odds ratio	lower 95% confidence interval	higher 95% confidence interval
149	0.00	--	--
147	0.48	0.20	1.14
145	0.53	0.36	0.77
143	1.18	0.77	1.80
141	*3.14*	*1.30*	*7.62*
139	4.02	0.25	64.72
137	0.30	0.11	0.84
135	*5.44*	*1.99*	*14.87*
133	--	--	--
131	--	--	--
125	--	--	--

Figure: Allele Frequencies of X Chromosomal Markers
Allele frequencies for each of the X chromosomal markers were analyzed. Significant differences in allele frequencies between cases and controls are highlighted by arrows.

(next page)


Allele frequencies for DXS1047
Frequencies
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0.4
0.3
0.2
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0
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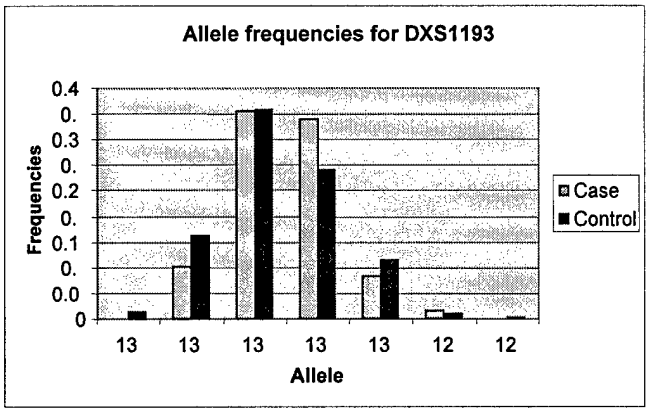
Alleles
Cases
Controls


Allele frequencies for DXS1193
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0.3
0.2
0.1
0
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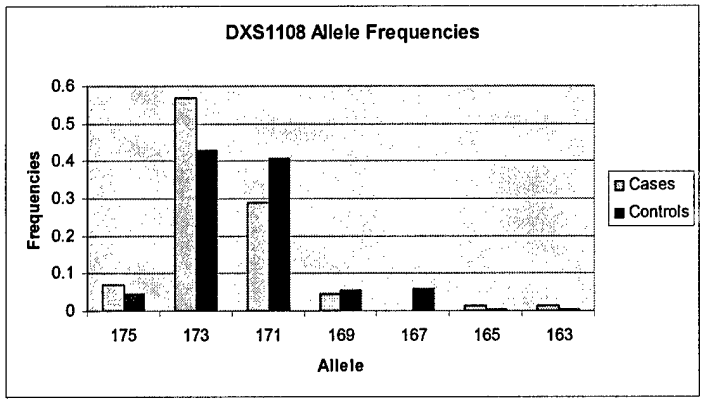
Allele
Case
Control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Frequencies
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0.2
0.1
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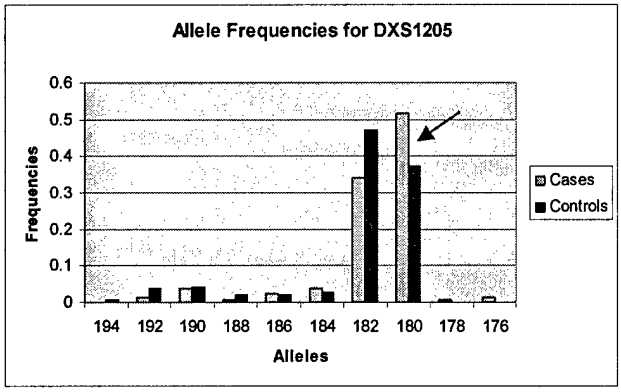
Allele
Cases
Controls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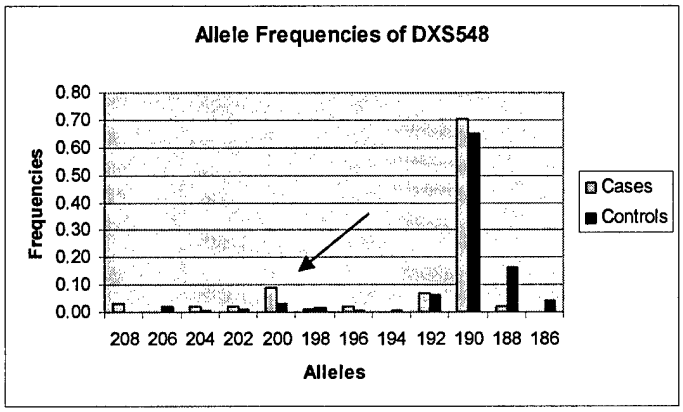
Alleles
Cases
Controls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0.00
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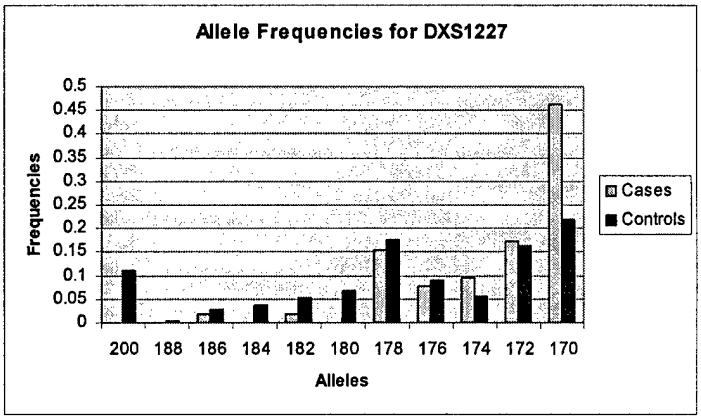
Alleles
Cases
Controls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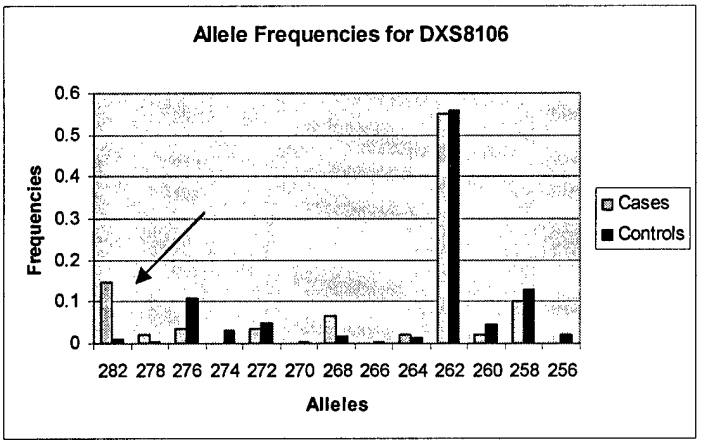
Alleles
Cases
Controls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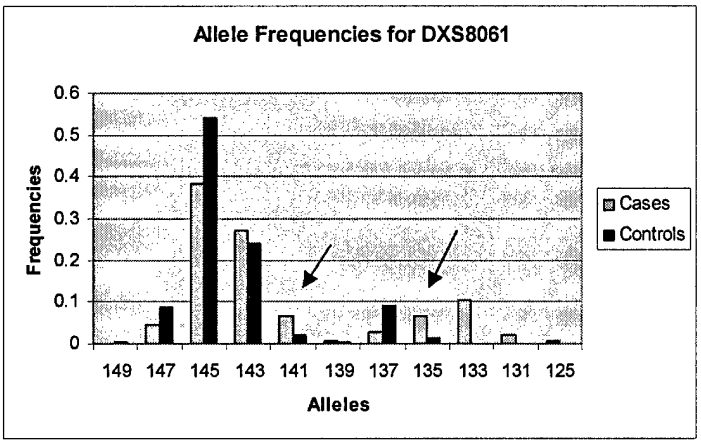
Alleles
Cases
Controls


Allele Frequencies for DXS8061
Frequencies
0.6
0.5
0.4
0.3
0.2
0.1
0
149 147 145 143 141 139 137 135 133 131 125
Alleles
Cases
Controls

KEY RESEARCH ACCOMPLISHMENTS:

Development of DNA databases from cases and controls for genomic analysis.

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

Identification of candidate loci for prostate cancer susceptibility on the X chromosome.

REPORTABLE OUTCOMES:

Proposal, "Genetic Susceptibility to Prostate Cancer in the Netherlands Cohort Study," (PC99-1496) was funded by USARMC.

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), was funded by NIH.

CONCLUSIONS

This work demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It has localized a candidate locus (candidate loci) on the X chromosome for prostate cancer susceptibility.

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